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ISOCITRICODEHYDROGENASE AND GLUTAMATE DEHYDROGENASE ACTIVITY OF GRANULOCYTES AND OF LYMPHOCYTES

/Following is the translation of an article by F. Belfiore and G. Calcara in the Italian-language periodical Bolletino della Societa Italiana di Biologia Sperimentale (Bulletin of the Italian Experimental Biology Society), Naples, Vol 39, No 23, 1963, pages 1571-1574-7

From the Pathological Medicine Institute of Catania University. Catania Section -- 26 July 1963 session.

Working on a research program intended to determine the enzyme picture of the leucocytes, we addressed ourselves to the problem of determining the activity of the individual categories of elements composing the leucocytary population, that is, basically, granulocytes and lymphocytes; we did this in order to obtain results relating to a homogeneous cell mass. We were quite aware of the difficulties encountered in isolating granulocytes and lymphocytes in a state of sufficient purity; to this end, we used a method (1, 2) which makes it possible for us to calculate the activity of these two types of cells on the basis of the enzyme activity of the leucocytes considered as a whole. Using this method, we were able to show in earlier research that the glucose-6-phosphate dehydrogenase activity (3), the arginase activity (1), the phosphoglycomutase and the pyruvatokinase activity (4) reveal a considerably higher activity in granulocytes than in lymphocytes. The glutamicoxalacetic transaminase activity (5) reveals the opposite behavior; here we have higher values for the lymphocytes; the aldolase, lacticodehydrogenase (6), malicodehydrogenase (7), succinodehydrogenase (8), phosphohexoseisomerase and acid phosphatase (9) activities reveal an equal degree of activity in the two types of elements. In this report we studied the glutamic hydrogenase and isocitricodehydrogenase activities of leucocytes; we used the previously mentioned method (1, 2) in order to get the granulocytes and lymphocyte activity. In connection with this topic, we noted the observations of Loehr (10), according to whom the two enzyme activities we considered in this article would appear to reveal a higher activity in the granulocytes.

Materials and methods. We studied the leucocytes of a group of normal subjects of both sexes between the ages of 20 and 65 years. The isocitricodehydrogenase activity (ICDH) was studied in 30 subjects, including 13 female subjects and 17 male subjects, using the method of Wolfson and Williams-Ashman (11); the results were expressed in units and were related to 1 ml of leucolysate diluted 1/400. The glutamic dehydrogenase activity (GLDH) was studied in 30 subjects, of whom 18 were males and 12 females; we determined this according to the method of Schmidt and his associates (12), relating the results, expressed in units, to 1 ml of leucolysate diluted 1/200.

Leucolysate was prepared by adding, to a volume of leucocytes, isolated by means of a method described elsewhere (1, 2), 399 volumes of distilled water for ICDH and 199 volumes for GLDH. This is why the data were related to a specific volume of leucocytes, rather than a specific number of leucocytes, as other authors have done. This was done for the purpose of relating the results to an always constant cellular mass, independently of the prevalence of the larger-volume leucocytes (granulocytes) or the smaller-volume (lymphocytes). When we, instead, work with leucocytary preparations with a constant number of elements, the data are related to a larger or smaller cellular mass, depending on whether the granulocytes or the lymphocytes prevail in the composition of the leucocytes. Once we have obtained the enzyme values for the leucolysate, as we did above, we calculated the activity of the granulocytes and the lymphocytes according to the method described elsewhere (1, 2).

Results. The results are shown in the following table and indicate that the glutamic dehydrogenase activity in the leucocytes reveals an average value of 5.52 U/ml of leucolysate diluted 1/200.) Using the procedure outlined above (1, 2), we were able to establish that this enzyme activity is contained primarily in the lymphocytes; this gives we an activity of 13.2 U/ml of leucolysate while in the granulocytes we have a rather low level amounting to 4.4 U/ml of leucolysate.

The difference between the activity of the two types of leucocytes is statistically significance (p < 0.05).

The isocitricodehydrogenase activity in the leucocytes revealed an average value of 176 U, in the granulocytes we had an average value of 109 U, and in the lymphocytes we had an average of 428 U. This enzyme activity likewise, however, reveals a definite difference between the granulocytes, and the lymphocytes, with higher values for the latter. This difference is statistically significant (p < 0.05). Our data agree with those observed by other authors (10) according to whom the two enzyme activities taken into consideration reveal a higher activity in the granulocytes than in the lymphocytes. This disagreement may in part be explained by the different procedure followed by these authors, as compared to our procedure. As a matter of fact, since these authors worked on leucocytary preparations containing a specific number of cells, it follows that, in the lymphocyte preparations, they worked on a cellular mass smaller than a similar preparation of granulocytes. This difference in cell mass may explain the low values obtained by these authors

ISOCITRICODEHYDROGENASE AND GLUTAMIC DEHYDROGENASE ACTIVITY OF LEUCOCYTES IN NORMAL SUBJECTS

(1)	(2)	(3)	(4)	(5)	(6)	(7)
			(8)			-
1	68	32	83,40	16,60	160	6,4
2	68	32	83,40	16,60	120	3,6
3	75	25	87,50	12,50	170	2,8
4	80	20	90,44	9,56	200	11,6
5	72	28	85,72	14,28	200	
6 7	77	23	88,78	11,22	90	
8	78 70	22 30	89,32 84,60	10,68	160 140	4,6
9	70	30 ·	84,60	15,40 15,40	160	1,2
1Ó	72	28	85,72	14,28	220	6,0
ii	74	26	87,10	12,90	280	6,0
12	75	25	87,50	12,50	180	6,2
13	68	32	83,40	16,60	160	6,4
14	68	32	83,10	16,60	100	4,4
15	89	11	95,00	5,00	110	4,0
16 .	68	, 32	83,40	16,60	250	6,4
17	68	32	83,40	16,60	120	4,6
18	90	10	95,50	4,50	-	4,0
19	62	38	79,38	20,62	-	5,8
20	70	30	84,61	15,36	-	3,6
9)	1		86	· 14	, 165 .	5,3
(10)(11)(12)					32, 34 12,70	2 0,5
	(1	2) 🛴 _			141-191	4,2-6,4
	•		(13)			
_,	65	35	71,38	19.69	120	4.8
1 2	60	40	78,00	13,62 22,00	130 120	3,4
2	65	35	71,38	18,62	200	3,4
4	5.3	47	62,61	27,39	. 200	3,2
5	63	37	80,00	20,00	250	
6	60	4G	78,00	22,00	250	8,0
7 /	48	57	64,00	36,00	240	7.2
8	. 38	62	59,00	41,00	160	8.2
9	, 61	39	71,50	18,50	350	8,0
10	57	43	75,70	24,30	150	8,0
11	55	. 45	74,20	25,80	110	4,8
12	64	46	80,74	19,26	90	10,8
13	48	52	68,50	31,50	80	9,0
14	47	53	68,56	31,44	•	7,6
15 16	56 ` 57	44	74,94 76,50	25,06 23,50		3,0 4,2
10 17	. 50	50	70,24	29,76		2,4
18	60 .	40	78,00	22,00		2,8
(9)		. —	72	28	187	5,8
(10)	(11) (12)				78,67 21,80	2,55 0,63
(14)	(1)) (11) (2)		•	144-230	4,6-7
((9)	,			109	4,4
	170	(11)			29 \$2-167	0,5 3,4-5,4
(15)	•)) (11) ₍₁	•		, .	-112
1-//	(9)	i		1	428	13,2
	130) i			711	43,7
		(111)			130	7,5
		\ //-	۵١		167-689	5,4-21

Legend: (1) case number; (2) number of granulocytes; (3) number of lymphocytes; (4) volume of granulocytes; (5) volume of lymphocytes; (6) ICDH activity; (7) GLDH activity; (8) cases in which granulocytes prevail; (9) mean; (10) standard deviation; (11) standard error; (12) confidence intervals; (13) cases where lymphocytes prevail; (14) granulocyte activity; (15) lymphocyte activity.

with respect to lymphocytes. Our finding of a higher glutamic dehydrogenase activity in the lymphocytes agrees with what one of us observed in earlier research (5) about the high content of the glutamic-oxalacetic transaminase lymphocytes, that is, another enzyme which, like glutamic dehydrogenase, acts on glutamate, a compound which however appears to have a particularly active metabolism in the lymphocytes. On the basis of the data given above we can say that we were able to confirm the existence of the definite difference between the enzyme picture of the granulocytes and lymphocytes which already turned up in our earlier research (1-8).

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